Monatshefte für Chemie - Chemical Monthly
https://doi.org/10.1007/s00706-018-2175-1

ORIGINAL PAPER

Kinetic studies of conjugate addition of amines to allenic and acrylic esters and their correlation with antibacterial activities against *Staphylococcus aureus*

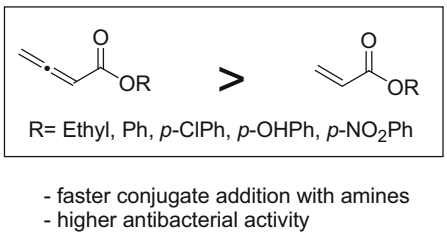
Cholpisit Kiattisewee[1] · Angkit Kaidad[1] · Chutima Jiarpinitnun[1] · Torsak Luanphaisarnnont[1]

Received: 12 November 2017 / Accepted: 12 February 2018

Abstract

Kinetic reactivities of various allenic and acrylic esters in conjugate addition reactions with various amines were investigated. Competition experiments showed that amines reacted selectively with allenic esters, which was also confirmed by quantitative determination of the rate constants. The antibacterial activity against *Staphylococcus aureus* of allenic and acrylic ester derivatives were also determined. Allenic esters were found to exhibit a higher antibacterial activity than its acrylic counterparts. A correlation between the kinetic property and the antibacterial activity suggested that a conjugate addition may involve in the antibacterial mechanism of these unsaturated esters.

Graphical abstract



Keywords Allenes · Alkenes · Kinetics · Antibiotics

Introduction

A conjugate addition is among the most important reactions in chemistry. The reaction has been used not only in organic synthesis [1–4], but also in the design of chemical probes for biological studies [5–8] as well as the design of potent therapeutics [9–13]. Therefore, it is crucial to investigate factors that play roles in efficiency of the reaction. One important factor is the nature of the electrophile in the reaction. Unsaturated esters such as acrylic [14–18] and allenic esters [19–23] have been shown to be effective electrophiles in conjugate addition reactions. Previous studies have shown that a double bond in allenes is thermodynamically more reactive than that of alkenes [24]. However, comparative kinetic behaviors of allenes and alkenes are less investigated [25–27]. To the best of our knowledge, the kinetic difference between alkenes and allenes in a conjugate addition reaction has not been explored. We envisioned that a better understanding of the difference in kinetics would promote further use of the

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00706-018-2175-1) contains supplementary material, which is available to authorized users.

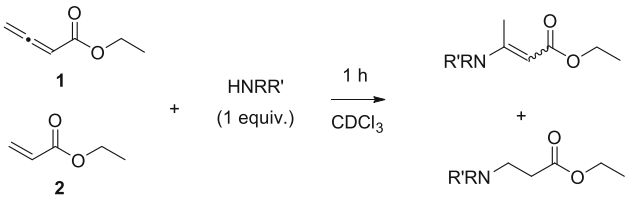
✉ Torsak Luanphaisarnnont
torsak.lua@mahidol.ac.th

[1] Department of Chemistry and Center of Excellence for Innovation in Chemistry (PERCH-CIC), Faculty of Science, Mahidol University, 272 Rama VI Road, Ratchathewi, Bangkok 10400, Thailand

Published online: 20 February 2018

conjugate addition to a greater extent. For instance, for therapeutic drugs that rely on the conjugate addition mechanism for their mode of action, knowledge in kinetic studies would facilitate the development of these drugs with enhanced potency [28]. A bioactive small molecule, 4-hydroxyphenyl acrylic ester, has previously been shown to exhibit antibacterial activity against *Staphylococcus aureus* (*S. aureus*) [29], a pathogenic Gram-positive bacterium that causes a widespread infection worldwide [30–32]. The antibacterial mechanism has been proposed to involve a conjugate addition reaction between acrylic ester and a nucleophile on bacterial surface [29]. Therefore, a modification of the conjugate accepting moiety from acrylic to allenic esters may improve their antibacterial activity. Comparison of the kinetic reactivity of conjugate addition and the antibacterial activity may support the proposed mechanism of this class of antibacterial small molecules. This research is aimed at investigating the kinetic difference between the allenic and acrylic esters in conjugate addition with amines and correlating the difference with their antibacterial activity.

Table 1 Competition experiments of ethyl allenic and acrylic esters with different amines



Entry	Amines	Conversion/%[a]	Ratio[b] (allenic:acrylic)
1	Aniline[c]	0	N.A.[d]
2	Benzylamine	< 5	N.A.[d]
3	*n*-Butylamine	< 5	N.A.[d]
4	Dibenzylamine	11	6:1
5	Diethylamine	58	> 20:1
6	Pyrrolidine	58	> 20:1
7	Piperidine	62	> 20:1

[a]Combined conversions of both acrylic and allenic esters

[b]Conversions of allenic ester:acrylic ester (determined by ^{1}H NMR)

[c]The reaction was performed and monitored for 6 h

[d]Not available

Results and discussion

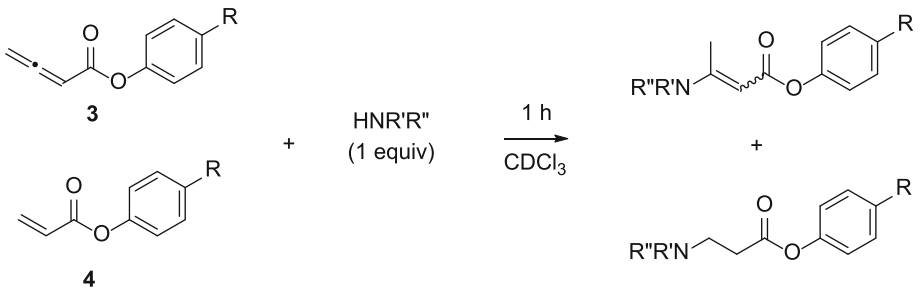
A competition experiment was first performed to study the difference between the kinetic reactivity of allenic and acrylic esters in conjugate addition reaction. Ethyl allenic ester and ethyl acrylic ester were used as model substrates. Various amines such as primary amines (aniline, benzylamine, and *n*-butylamine), secondary amines (dibenzylamine and diethylamine), and cyclic amines (pyrrolidine and piperidine) were used as a nucleophile. The ratios of the conversions of the starting esters were calculated and used to determine the relative reactivities of the two esters. No secondary addition to the conjugated product of allenic ester was observed in the reaction.

The result, shown in Table 1, suggested that ethyl allenic ester (**1**) reacted faster with all amine nucleophiles than ethyl acrylic ester (**2**). It is also noteworthy that a primary amine reacts very slowly, giving only trace amount (< 5%) of product (entries 2, 3) when compared with a secondary amine (entries 4–7). Aniline, an aromatic primary amine, did not give any conjugate addition product even after 6 h (entry 1), which agreed well with the previously reported low nucleophilicity of aniline in aprotic organic solvent [33].

Competition experiments between aromatic allenic esters and aromatic acrylic esters with various substituents were also investigated (Table 2). Esters with different substituents on the aromatic ring—including phenyl allenic ester (**3a**), 4-nitrophenyl allenic ester (**3b**), 4-hydroxyphenyl allenic ester (**3c**), 4-chlorophenyl allenic ester (**3d**), phenyl acrylic ester (**4a**), 4-nitrophenyl acrylic ester (**4b**), 4-hydroxyphenyl acrylic ester (**4c**), 4-chlorophenyl acrylic ester (**4d**)—were used as model substrates. Similar to the result of ethyl esters, no secondary addition into the conjugate addition product of aromatic allenic esters was observed. In all cases of primary amines and secondary amines, allenic esters underwent the conjugate addition reaction at a faster rate than acrylic esters. Phenyl allenic ester (**3a**) showed exceptionally high selectivities toward both primary and secondary amines (16:1 to 20:1) over phenyl acrylic ester (**4a**).

The qualitative comparison of the kinetic reactivity of allenic and acrylic esters in conjugate addition suggested that allenic ester reacted faster than acrylic esters. We further pursued the quantitative determination of the rate constant of the reaction using a pseudo-first-order approach. Ethyl allenic ester (**1**) and ethyl acrylic ester (**2**) were chosen as the representatives for allenic and acrylic esters, respectively. Preliminary result suggested that addition of primary amines to allenic esters initially gave an *E* product, which was gradually converted to a more stable *Z* product. The presence of these kinetic and thermodynamic products complicated the kinetic investigation; therefore, a secondary amine was used as a model substrate. Pyrrolidine was chosen as a representative for an amine nucleophile because the reaction with pyrrolidine gave only a *Z* product, which simplified the kinetic investigation. The concentration of the conjugate acceptor was monitored using ^{1}H NMR spectroscopy. The linear

Table 2 Competition experiments of aryl allenic and acrylic esters with amines



Entry	Amines	R=H (**a**)		R=NO_2 (**b**)		R=OH (**c**)		R=Cl (**d**)	
		Conv./%[a]	Ratio[b]	Conv./%[a]	Ratio[b]	Conv./%[a]	Ratio[b]	Conv./%[a]	Ratio[b]
1	Aniline[c]	14	20:1	33	5:1	27	2:1	16	10:1
2	Benzylamine	61	20:1	88	11:1	55	20:1	58	20:1
3	*n*-Butylamine	58	20:1	75	11:1	47	8:1	32	11:1
4	Dibenzylamine	53	20:1	80	13:1	56	20:1	56	20:1
5	Diethylamine	51	20:1	63	11:1	56	16:1	40	20:1
6	Pyrrolidine	83	16:1	95	7:1	98	8:1	75	9:1
7	Piperidine	70	19:1	83	3:1	90	4:1	72	6:1

[a]Combined conversions of both acrylic and allenic esters

[b]Conversions of allenic ester:acrylic ester (determined by ^{1}H NMR)

[c]The reaction was performed and monitored for 6 h

graph-fitting was used to determine the observed rate (Fig. 1a). The observed rates at various excess concentrations of amine were used to determine the actual rate of the reaction. The rate constant for ethyl allenic ester (**1**) was 2.99×10^{-1} M^{-1} s^{-1} (Fig. 1b) while that for ethyl acrylic ester (**2**) was 2.33×10^{-3} M^{-1} s^{-1} (Fig. 1c), approximately 100-fold slower. These rate constants verified that allenic esters react faster in a conjugate addition reaction than acrylic esters. The results correlate with the qualitative result from the competition experiments.

As the mode of action of 4-hydroxyphenyl acrylic ester in its antibacterial activity was suggested to involve the ability of bacterial nucleophile to undergo conjugate addition to the unsaturated vinyl moiety [29], we further investigated whether the significant difference in kinetic behavior between allenic and acrylic ester would play a role in antibacterial activities. We performed susceptibility tests of allenic esters in comparison with acrylic esters against *S. aureus*. A library of phenyl allenic esters and phenyl acrylic esters with various substituents on phenyl ring were synthesized. We first performed qualitative Kirby-Bauer disk diffusion method to screen for antibacterial activity against *S. aureus* strain ATCC 25923 (Table 3). The result suggested the vital role of unsaturation in the observed antibacterial activity (entries 1 and 2 vs. entries 3 and 4). In addition, the aromatic ring is also important in the antibacterial activity—aliphatic unsaturated esters (entries 5 and 6) showed no inhibition zones when compared with aromatic unsaturated esters.

We further performed a quantitative microdilution assay with *S. aureus* strain ATCC 29213 to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The results are also shown in Table 3. The MICs and MBCs of aryl allenic esters are lower than those of the aryl acrylic counterparts with an exception of the MBC of 4-nitrophenyl allenic ester (entry 9), which was comparable to that of the 4-nitrophenyl acrylic ester (entry 10). As suggested by their MICs and MBCs values, aryl allenic esters exhibited more potent antibacterial activity against Gram-positive *S. aureus* than aryl acrylic esters.

There is a correlation between the antibacterial activity and the kinetic property of these small antibacterial molecules. When compared with phenyl acrylic ester (**4a**), phenyl allenic ester (**3a**) with higher kinetic reactivity in conjugate addition reaction also showed higher antibacterial activity (Table 4, entry 3 vs. entry 4). It is noteworthy that aryl allenic esters with antibacterial activities showed higher rates of conjugate addition than other esters (entries 3 and 5–7 vs. entries 1, 2, and 4). This trend suggested that a mechanism of action of this class of compounds may involve a conjugate addition.

We further investigated whether the substitutions on the benzene ring affect kinetic behavior and antibacterial

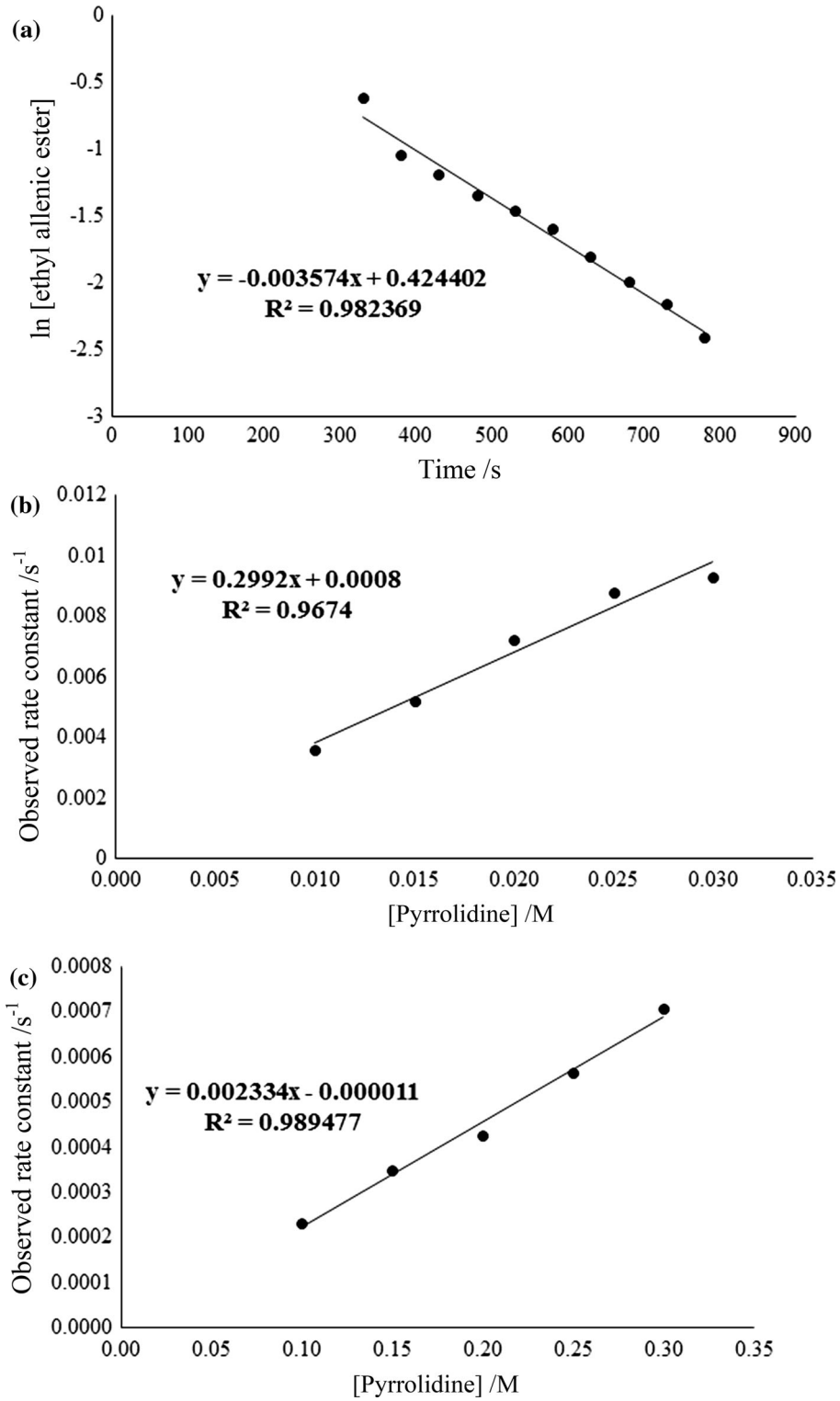
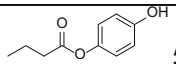
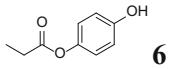
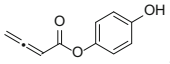
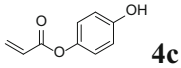
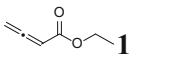
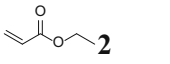
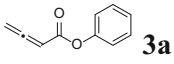
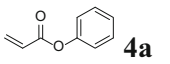
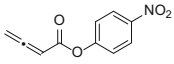
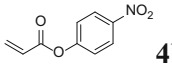
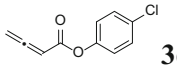
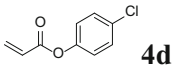


Fig. 1 Representatives of the linear graph-fitting in rate determination: **a** observed rate constant (addition of 10 equiv. of pyrrolidine to ethyl allenic ester (**1**); **b** actual rate constant of ethyl allenic ester (**1**); **c** actual rate constant of ethyl acrylic ester (**2**)

activity of aryl allenic esters. Selected aryl allenic esters with different substituents (phenyl, 4-nitrophenyl, 4-hydroxyphenyl, and 4-chlorophenyl) were used to determine the rates of conjugate addition. We observed that the antibacterial activity of the allenic esters could be influenced by the substitution on the benzene ring. Further

Table 3 Antibacterial activities of allenic and acrylic esters determined by disk diffusion[a] and microdilution assay[b]

Entry	Compound	Inhibition zone/mm	MIC/mM	MBC/mM
1	 **5**	9.57 ± 0.33	10.0	10.0
2	 **6**	7.22 ± 0.57	> 10.0	> 10.0
3	 **3c**	23.35 ± 0.39	0.625	0.938
4	 **4c**	20.13 ± 1.10	0.938	1.25
5	 **1**	6.00	N.D.[c]	N.D.[c]
6	 **2**	6.00	N.D.[c]	N.D.[c]
7	 **3a**	15.93 ± 0.63	1.25	2.50
8	 **4a**	6.00	N.D.[c]	N.D.[c]
9	 **3b**	14.36 ± 1.36	0.469	2.50
10	 **4b**	15.67 ± 0.43	0.625	2.50
11	 **3d**	26.85 ± 0.59	2.50	5.00
12	 **4d**	10.68 ± 0.38	5.00	10.0

[a]2.5 μmol of test compounds in DMSO were used for disk diffusion testing

[b]Each compound was twofold diluted based on its stock concentration

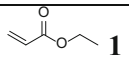
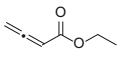
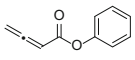
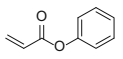
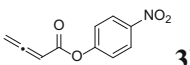
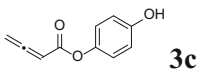
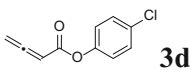
[c]Not determined due to no inhibition zone in disk susceptible diffusion test

investigation on the role of substitution on antibacterial activity is undergoing.

Conclusion

In summary, we found that allenic esters underwent a conjugate addition of amine nucleophiles faster than that of acrylic esters. Aryl allenic esters also exhibited higher antibacterial activity than aryl acrylic esters. There is a correlation between the rate of a conjugate addition of a compound and its antibacterial activity, which implies that a conjugate addition reaction may be an important step in their mechanism of antibacterial activity. Further studies to elucidate the mechanism of action of this class of antibacterial compounds and a structural modification of other bioactive compounds to include an allene moiety are currently ongoing and will be reported in due course.

Table 4 A correlation between rate of conjugate addition and antibacterial activity of selected allenic and acrylic esters

Entry	Allenic ester	Rate constant[a]/ M^{-1} s^{-1}	MIC/mM	MBC/mM
1	 **1**	0.00233[b]	N.D.[c]	N.D.[c]
2	 **2**	0.258 (0.299)[b]	N.D.[c]	N.D.[c]
3	 **3a**	3.01	1.25	10.0
4	 **4a**	0.0184[b]	N.D.[c]	N.D.[c]
5	 **3b**	12.3	0.469	2.50
6	 **3c**	2.59	0.625	0.938
7	 **3d**	5.96	2.50	5.00

[a]Rate constants of conjugate addition of pyrrolidine determined by UV spectroscopy

[b]Rate constants of conjugate addition of pyrrolidine determined by NMR spectroscopy

[c]Not determined

Experimental

Unless specified otherwise, all reactions were performed under an ambient atmosphere in oven-dried glassware with magnetic stirring. Reactions conducted below ambient temperature were cooled by external ice water bath for 0 °C. Reactions above ambient temperature were heated by a silicone oil bath.

Purification of reaction products was carried out on silica gel (SiO_2; 60 Å silica gel, Merck Grade, 70–230 Mesh). Analytical thin-layer chromatography was performed on Merck TLC alumina sheet pre-coated with silica gel 60 F254 plates. Visualization was accomplished with UV light and aqueous potassium permanganate.

Commercial reagents were purchased from Sigma Aldrich, TCI, Merck, and Alfa Aesar and used as received unless otherwise noted.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AVANCE 400 spectrometer (400 MHz) in $CDCl_3$. Chemical shifts are reported in ppm from tetramethylsilane with the residual solvent resonance as an internal standard ($CDCl_3$ at 7.26 ppm). Data are reported as follows: (br = broad, app = apparent, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet; coupling constant(s) in Hz, integration). Proton-decoupled carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Bruker AVANCE 400 (100 MHz) spectrometer in $CDCl_3$. Chemical shifts are reported in ppm from tetramethylsilane with the residual solvent resonance as an internal standard ($CDCl_3$ at 77.0 ppm). Infrared spectra were recorded as a neat compound on a Bruker ALPHA FT-IR spectrometer, and only partial data were listed. High-resolution mass spectra were obtained on a Bruker micro TOF spectrometer in the ESI mode.

General procedure for preparations of aryl esters

To a suspension of 562 mg 2-chloropyridinium iodide (2.2 mmol, 1.1 equiv) and 612 mg potassium carbonate

(4.4 mmol, 2.2 equiv) in 20 cm^3 dichloromethane, the corresponding acid (2.2 mmol, 1.1 equiv) and the corresponding phenol (2.0 mmol, 1.0 equiv) were added. The reaction was stirred at room temperature for 4 h. The reaction mixture was then filtered through a silica plug, washed with dichloromethane, and concentrated. The residue was purified by column chromatography to yield the desired product.

Phenyl 2,3-butadienoate (3a) The title compound was synthesized according to general procedure with 185 mg 3-butynoic acid (2.2 mmol) and 188 mg phenol (2.0 mmol) as starting materials. The product was purified by flash column chromatography (5–10% EtOAc in hexanes) as a colorless oil (yield 180 mg, 56%). The ^{1}H NMR was found to be identical to the published data [34].

4-Nitrophenyl 2,3-butadienoate (3b, $C_{10}H_7NO_4$) The title compound was synthesized according to general procedure with 185 mg 3-butynoic acid (2.2 mmol) and 278 mg 4-nitrophenol (2.0 mmol) as starting materials. The product was purified by flash column chromatography (10–20% EtOAc in hexanes) as a white solid (yield 127 mg, 31%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.28$ (d, $J = 9.2$ Hz, 2H), 7.33 (t, $J = 9.2$ Hz, 2H), 5.84 (t, $J = 6.4$ Hz, 1H), 5.40 (t, $J = 6.5$ Hz, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 217.1$, 163.1, 155.4, 145.2, 125.1, 122.3, 87.1, 80.2 ppm; IR (neat): $\bar{\nu} = 3118$, 3074, 3042, 2998, 1965, 1743, 1592, 1515, 1489, 1344, 1314, 1216 cm^{-1}; HRMS (ESI): $m/z = 228.0270$; 228.0273 calculated for $C_{10}H_7NNaO_4$ ($[M + Na^+]$).

4-Hydroxyphenyl 2,3-butadienoate (3c, $C_{10}H_8O_3$) The title compound was synthesized according to general procedure with 185 mg 3-butynoic acid (2.2 mmol) and 220 mg hydroquinone (2.0 mmol) as starting materials using acetonitrile as a solvent. The product was purified by flash column chromatography (10–20% EtOAc in hexanes) as a colorless oil (yield 28 mg, 8%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.97$ (d, $J = 8.9$ Hz, 2H), 6.79 (t, $J = 8.9$ Hz, 2H), 5.81 (t, $J = 6.5$ Hz, 1H), 5.32 (t, $J = 6.5$ Hz, 2H), 5.30 (s, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 217.1$, 163.1, 155.4, 145.2, 125.1, 122.3, 87.1, 80.2 ppm; IR (neat): $\bar{\nu} = 3407$, 3071, 3038, 2990, 2963, 2920, 1968, 1705, 0601, 1508, 1446, 1421, 1342, 1231, 1186, 1127 cm^{-1}; HRMS (ESI): $m/z = 199.0372$; 199.0371 calculated for $C_{10}H_8NaO_3$ ($[M + Na]^+$).

4-Chlorophenyl 2,3-butadienoate (3d, $C_{10}H_7ClO_2$) The title compound was synthesized according to general procedure with 185 mg 3-butynoic acid (2.2 mmol) and 257 mg 4-chlorophenol (2.0 mmol) as starting materials. The product was purified by flash column chromatography (5–10% EtOAc in hexanes) as a white solid (yield 144 mg, 37%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.34$ (d, $J = 8.9$ Hz, 2H), 7.07 (t, $J = 8.9$ Hz, 2H), 5.81 (t, $J = 6.5$ Hz, 1H), 5.35 (d, $J = 6.5$ Hz, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 216.7$, 163.9, 149.2, 131.2, 129.4, 122.9, 87.4, 79.9 ppm; IR (neat): $\bar{\nu} = 3098$, 3038, 3072, 2990, 1970, 1721, 1487, 1420, 1405, 1322, 1204, 1167, 1126, 1086 cm^{-1}; HRMS (ESI): $m/z = 217.0033$; 217.0032 calculated for $C_{10}H_7ClNaO_2$ ($[M + Na]^+$).

Phenyl acrylate (4a) The title compound was synthesized according to general procedure with 158 mg acrylic acid (2.2 mmol) and 188 mg phenol (2.0 mmol) as starting materials. The product was purified by flash column chromatography (5–10% EtOAc in hexanes) as a colorless oil (yield 104 mg, 35%). The ^{1}H NMR was found to be identical to the published data [29].

4-Nitrophenyl acrylate (4b) The title compound was synthesized according to general procedure with 158 mg acrylic acid (2.2 mmol) and 278 mg 4-nitrophenol (2.0 mmol) as starting materials. The product was purified by flash column chromatography (10–20% EtOAc in hexanes) as a white solid (yield 202 mg, 32%). The ^{1}H NMR was found to be identical to the published data [35].

4-Hydroxyphenyl acrylate (4c) The title compound was synthesized according to general procedure with 158 mg acrylic acid (2.2 mmol) and 220 mg hydroquinone (2.0 mmol) as starting materials using acetonitrile as a solvent. The product was purified by flash column chromatography (10-20% EtOAc in hexanes) as a colorless oil (yield 17 mg, 5%). The ^{1}H NMR was found to be identical to the published data [29].

4-Chlorophenyl acrylate (4d, $C_9H_7ClO_2$) The title compound was synthesized according to general procedure with 158 mg acrylic acid (2.2 mmol) and 257 mg 4-chlorophenol (2.0 mmol) as starting materials. The product was purified by flash column chromatography (5–10% EtOAc in hexanes) as a colorless oil (yield 121 mg, 33%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.35$ (d, $J = 9.2$ Hz, 2H), 7.08 (d, $J = 9.2$ Hz, 2H), 6.61 (dd, $J = 17.3$, 1.0 Hz, 1H), 6.31 (dd, $J = 17.3$, 10.5 Hz, 1H), 6.03 (dd, $J = 10.4$, 1.0 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 164.2$, 149.0, 133.0, 131.2, 129.4, 127.5, 122.9 ppm; IR (neat): $\bar{\nu} = 3106$, 3068, 3043, 1739, 1635, 1486, 1404, 1293, 1248, 1198, 1143 cm^{-1}; HRMS (ESI): $m/z = 205.0037$; 205.0032 calculated for $C_9H_7ClNaO_2$ ($[M + Na]^+$).

4-Hydroxyphenyl butanoate (5) The title compound was synthesized according to general procedure with 194 mg butanoic acid (2.2 mmol) and 220 mg hydroquinone (2.0 mmol) as starting materials using acetonitrile as a solvent. The product was purified by flash column chromatography (10–20% EtOAc in hexanes) as a colorless oil

(yield 115 mg, 32%). The ^{1}H NMR was found to be identical to the published data [36].

4-Hydroxyphenyl propanoate (6) The title compound was synthesized according to general procedure with 163 mg propanoic acid (2.2 mmol) and 220 mg hydroquinone (2.0 mmol) as starting materials using acetonitrile as a solvent. The product was purified by flash column chromatography (10–20% EtOAc in hexanes) as a colorless oil (yield 30 mg, 9%). The ^{1}H NMR was found to be identical to the published data [29].

Competition experiments of allenic and acrylic esters

The prepared stock solutions of allenic esters, acrylic esters, aniline, benzylamine, dibenzylamine, pyrrolidine, piperidine, *n*-butylamine, and diethylamine were prepared at 60 mM in $CDCl_3$. 1,3,5-Trimethoxybenzene was prepared as an internal standard at 20 mM in $CDCl_3$. To an NMR tube, 100 mm^3 of the allenic ester stock solution, 100 mm^3 of the acrylic ester stock solution, 100 mm^3 of 1,3,5-trimethoxybenzene stock solution, and 200 mm^3 of $CDCl_3$ were added. The selected amine stock solution (100 mm^3) was then added to the NMR tube, making the final concentration of the allenic ester, the acrylic ester, and the amine 10 mM. The NMR data were collected at 1 and 6 h after addition of amine nucleophiles. The concentrations of allenic and acrylic esters at the collected time were calculated by their integration with respect to the internal standard.

NMR kinetic experiments

Stock solutions of ethyl allenoate (6 mM), ethyl acrylate (60 mM), and pyrrolidine (60 mM and 600 mM in $CDCl_3$) were prepared. A stock solution of 1,3,5-trimethoxybenzene (20 mM) in $CDCl_3$ was prepared as an internal standard. To an NMR tube, the stock solution of the selected ester (100 mm^3) and the stock solution of the internal standard (100 mm^3) were added. $CDCl_3$ was then added to the reaction mixtures at different quantity (Table S-1). A solution of pyrrolidine (60 mM) was then added to the allenic ester solution to give 600 mm^3 of the reaction mixture with excess pyrrolidine (10 ×–30 ×) (Table S-1). The reaction mixture was immediately subjected to NMR quantification, and the concentration of starting ester and conjugate addition product was calculated by their integrations with respect to the internal standard for each collection time. The data were processed to give pseudo-first-order rate constant for each reaction condition. Ethyl acrylate kinetic experiments were performed in the same manner with different starting concentration; 60 mM for ethyl acrylate and 600 mM for pyrrolidine.

UV kinetic experiments

Stock solutions of ethyl allenoate (150 μM) and pyrrolidine (1.50–4.50 mM) were prepared prior usage in the solvent of choice. To a UV cuvette, 1 cm^3 each of solvent, ethyl allenoate solution and pyrrolidine solution was added, respectively. The scanning spectrum was recorded in kinetics mode of Agilent Cary-60 UV–Visible spectrophotometer. The absorbance at 290 nm was used for pseudo-first-order rate constant determination of each condition (10 ×–30 ×). The rate constant determination of other allenic esters was performed in the same manner.

Microbiology

The turbidity was measured using Wallac Victor V1420 Multilabel HTS Counter with rotating filter wheel B, at $\lambda = 595$ nm in the photometry mode. The diameter of inhibitory zones was measured using digital sliding calipers. Muller–Hinton broth, Tryptic soy broth, and Bacto agar are from BD (Spark, MD). *Staphylococcus aureus* ATCC 29213 and ATCC 25923 were received as gifts from Cornell University Food Safety Lab.

Bacterial strains and inoculums preparation

Allenic esters and acrylic esters were tested against *Staphylococcus aureus* ATCC 29213 and ATCC 25923 in antimicrobial disk diffusion susceptibility test and microdilution test, respectively. A single colony of each strain was picked and transferred into 5 cm^3 of Tryptic soy broth (TSB) in a 15-cm^3 test tube, which was then capped and incubated at 37 °C for 15–18 h. The suspension was subsequently diluted in phosphate buffer saline (PBS) to yield the appropriate inoculum density of 8×10^7 CFU/cm^3 for disk diffusion susceptibility test and 2×10^6 CFU/cm^3 for microdilution assay. The inoculum was used within 15 min after adjusting the density. Note that the phosphate buffer saline (PBS) was prepared by dissolving NaCl (137 mM), KCl (2.1 mM), $Na_2HPO_4{\cdot}7H_2O$, and KH_2PO_4 (2 mM) in 1000 cm^3 of H_2O. The pH of the resulting solution was adjusted 7.4 and autoclaved at 121 °C for 15 min.

Disk diffusion susceptibility test (Kirby-Bauer method) [37, 38]

Muller–Hinton agar (MHA) was prepared based on the supplier instruction and autoclaved to sterilize. The sterilized agar was poured into 90-mm Petri dishes to acquire

approximately 4 mm in thickness. After solidification, tested strains were spread on the agar surface using sterile swap. The spread inoculum was approximately 8×10^7 CFU/cm^3. The inoculated plate was allowed to stand at room temperature for 3–10 min before the application of disks (disks should be applied to the surface of agar plate within 15 min). Subsequently, small filter paper disks (6 mm) were placed on the bacterial spread agar plate, and a solution of test compounds (2.5 μmol) diluted in DMSO was transferred onto each paper disk. After 18–20 h of incubation at 37 °C in ambient air incubator, the diameters of inhibitory zones were measured in millimeters using sliding calipers. The experiments were performed at least three times.

For MHA plate, the zone diameter was measured from the back of the plate. The inhibition zones were classified as complete and incomplete inhibition. The complete zone margin is identified as the area where no visible growth can be observed by the naked eyes.

Microdilution method of antimicrobial susceptibility test [37]

The stock solutions of synthetic compounds, which exhibited an inhibition zone in the disk diffusion assay, were prepared in DMSO. The stock solutions were doubly diluted in sterilized Muller–Hinton broth (MHB) to obtain the required concentrations. A 180 mm^3 aliquot of each compound at different dilutions was transferred to sterilized 96-well plates. To the aliquot of tested compounds, a 20 mm^3 of test strain suspension, whose inoculum density was adjusted to 2×10^5 CFU/cm^3, was added to each well. The plates were incubated at 37 °C for 20 h. After incubation, the optical density of each well was determined using a microplate reader. The minimum inhibitory concentration (MIC) was determined as the lowest concentration with no visible growth (indicated by basal optical density). The experiments were performed at least three times.

Minimum bactericidal concentration test [37]

Based on the results from microdilution test, the solution of a well that appeared clear or qualitatively exhibited basal optical density was spread on TSA agar plate. The plate was subsequently incubated at 37 °C for 18–20 h. The lowest concentration that shows no colony is recorded as the minimum bactericidal concentration.

Acknowledgements This research was supported by Thailand Research Fund (Grant MRG5980118), the Faculty of Science, Mahidol University, the National Research Universities Initiative, and the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Office of the Higher Education Commission, Ministry of Education (OHEC). C. K. thanks the Sri Trang Thong Scholarship and the Young Scientist Scholarship program, and A. K. thanks the Young Scientist Scholarship program for financial support. We also thank the Department of Chemistry and the Central Instrumental Facility (CIF) at the Faculty of Science, Mahidol University for research facilities.

References

1. Perlmutter P (1992) Conjugate addition reactions in organic synthesis. Pergamon Press, Oxford
2. Rossiter BE, Swingle NM (1992) Chem Rev 92:771
3. Hayashi M, Matsubara R (2017) Tetrahedron Lett 58:1793
4. Phelan JP, Ellman JA (2016) Beilstein J Org Chem 12:1203
5. Du J, Yang Z, Qi H, Yang XF (2011) Luminescence 26:486
6. Kalia D, Malekar PV, Parthasarathy M (2016) Angew Chem Int Ed 55:1432
7. Lin W, Yuan L, Cao Z, Feng Y, Long L (2009) Chem Eur J 15:5096
8. Dai X, Wu QH, Wang PC, Tian J, Xu Y, Wang SQ, Miao JY, Zhao BX (2014) Biosens Bioelectron 59:35
9. Jackson PA, Widen JC, Harki DA, Brummond KM (2017) J Med Chem 60:839
10. Klaic L, Trippier PC, Mishra RK, Morimoto RI, Silverman RB (2011) J Am Chem Soc 133:19634
11. Lopachin RM, Gavin T, DeCaprio A, Barber DS (2012) Chem Res Toxicol 25:239
12. Chu W, Rothfuss J, d'Avignon A, Zeng C, Zhou D, Hotchkiss RS, Mach RH (2007) J Med Chem 50:3751
13. Gotz MG, James KE, Hansell E, Dvorak J, Seshaadri A, Sojka D, Kopacek P, McKerrow JH, Caffrey CR, Power JC (2008) J Med Chem 51:2816
14. House HO, Schellenbaum M (1963) J Org Chem 28:34
15. Dieter RK, Velu SE (1997) J Org Chem 62:3798
16. Bandyopadhyay D, Mukherjee S, Turrubiartes LC, Banik BK (2012) Ultrason Sonochem 19:969
17. Guo SH, Xing SZ, Mao S, Gao YR, Chen WL, Wang YQ (2014) Tetrahedron Lett 55:6718
18. Kuwajima I, Murofushi T, Nakamura E (1976) Synthesis 1976:602
19. Abbas A, Xing B, Loh TP (2014) Angew Chem Int Ed 53:7491
20. Paik YH, Dowd P (1986) J Org Chem 51:2910
21. Waizumi N, Itoh T, Fukuyama T (1998) Tetrahedron Lett 39:6015
22. Harvey GR, Ratts KW (1966) J Org Chem 31:3907
23. Ibrahim-Ouali M, Sinibaldi ME, Troin Y, Cuer A, Dauphin G, Gramain JC (1995) Heterocycles 41:1939
24. Krause N, Hashmi ASK (2004) Modern allene chemistry. Wiley-VCH Verlag, Weinheim
25. Atkinson R, Perry RA, Pitts JN (1977) J Chem Phys 67:3170
26. Ito O (1993) J Org Chem 58:1466
27. Luanphaisarnnont T (2014) Tetrahedron Lett 55:6803
28. Krenske EH, Petter RC, Houk KN (2016) J Org Chem 81:11726
29. Kim JH, Park ES, Shim JH, Kim MN, Moon WS, Chung KH, Yoon JS (2004) J Agric Food Chem 52:7480
30. Chambers HF, DeLeo FR (2009) Nat Rev Micro 7:629
31. Thomer L, Schneewind O, Missiakas D (2016) Annu Rev Patho Mech 11:343
32. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG (2015) Clin Microbiol Rev 28:603
33. Kanzian T, Nigst TA, Maier A, Pichl S, Mayr H (2009) Eur J Org Chem 2009:6379
34. Cowen BJ, Saunders LB, Miller SJ (2009) J Am Chem Soc 131:6105

35. Barr L, Lincoln SF, Easton CJ (2006) Chem Eur J 12:8571
36. Jankowiak A, Kaszynski P, Tilford WR, Ohta K, Januszko A, Nagamine T, Endo Y (2009) Beilstein J Org Chem 5:14
37. Schwalbe R, Lynn SM, Goodwin AC (2007) Anti-microbial susceptibility testing protocols. CRC Press, Boca Raton
38. Andrews JM (2007) J Antimicrob Chemother 60:20